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EXPRESSION OF G PROTEIN-COUPLED RECEPTOR KINASE SUBTYPES IN THE HUMAN URINARY BLADDER DETRUSOR MUSCLE IN THE NORMAL AND OBSTRUCTIVE BLADDER -A POSSIBLE MECHANISM FOR OVERACTIVE BLADDER-

Aims of Study

In overactive bladder, defined as symptoms of frequency, urgency, and urge incontinence, the one without evident neuropathy is called unstable bladder or bladder instability. Muscarinic acetylcholine receptor (M) antagonist would be a useful tool for the pharmacological treatment of unstable bladder, because human urinary bladder contraction is mediated by autonomic nervous system, mostly via cholinergic innervation of muscarinic acetylcholine receptors. Mechanism that may cause the smooth muscle of the detrusor to become less sensitive to incoming stimuli could have serious implications for the regulation of normal bladder physiology and certain pathological conditions. Desensitization of muscarinic acetylcholine receptor is one of such mechanisms, and is mediated by phosphorylation of muscarinic acetylcholine receptor by G protein-coupled receptor kinases (GRKs). M1, M2 and M3 receptors might be phosphorylated by GRK2 and GRK3. This mechanism could be related to the sthenia and asthenia of urinary bladder activity. In the present study, we examine the expression of mRNA of GRK subtypes and muscarinic acetylcholine receptor subtypes in the detrusor smooth muscle of the human urinary bladder. Furthermore, we confirm the presence and the localization of GRK proteins in the detrusor smooth muscle of the human normal urinary bladder and urinary outlet obstructive bladder with benign prostatic hyperplasia (BPH). Full informed consent was obtained from each patient.

Methods

Detrusor smooth muscle tissues of the human urinary bladder were obtained from 12 patients. Six patients, who underwent radical cystectomy due to bladder cancer, did not have bladder outlet obstruction. The other 6 patients, who underwent open prostatectomy due to BPH, had bladder outlet obstruction. Portions of the dome or anterior wall without macroscopically malignant lesion were used for the study. RT-PCR followed by Southern hybridization was performed using total RNA extracted from human urinary bladder detrusor, oligonucleotide primers specific for GRK2, M2, M3 and ß-actin. Antibodies to GRK2, GRK3 and GRK4 were used to confirm the presence of the protein product in the human urinary bladder. Primary antibody was detected by means of the ABC method for immunohistochemical staining. Proteins extracted from human urinary bladder detrusor were electrophoresed and blotted onto a membrane by western blotting technique. Immunoreactive proteins were detected and visualized by a chemiluminescence reagent.

Results

All DNAs transcribed from 4 different mRNAs (M2, M3, GRK2, and ß-actin) were successfully amplified and size-fractionated by 3.0% agarose gel. Southern blotting hybridization of these electrophoresed DNAs revealed positive single band. PCR with non-reverse transcribed RNA or PCR without cDNA revealed no amplification of DNA, suggesting absence of genomic DNA contamination. Immunohistochemical staining for GRK2 was clearly found in the detrusor smooth muscle of the human urinary bladder, but it was less effectively found in the detrusor smooth muscle of the outlet obstractive bladder with BPH than that of normal urinary bladder. No staining was observed in consecutive sections processed with the antibody preadsorbed against the immunizing peptide. Staining levels for GRK3 and GRK4 in the normal bladder detrusor were very low and middle, respectively. Antibodies specific for GRK2, GRK3 or GRK4 were produced and characterized by Western blotting analysis. Among the three proteins of GRKs, the signal to GRK2 (79 kDa) was the strongest. But the band of GRK2 in obstructive bladder was also significantly weakened in comparison with normal bladder. The signal to actin (42 kDa) was equally detected in each lane. The band of GRK3 was very weak,

but was slightly stronger in obstructive bladder than in normal bladder. The expression of GRK4 protein was moderate in both normal and obstructive bladder.

Conclusions

This study has demonstrated that the expression of GRK2 protein is strong in the human bladder detrusor, but is significantly weakened in obstructive bladder in comparison with normal bladder. Diminution for desensitization of muscarinic acetylcholine receptors could reinforced acetylcholine-induced bladder contraction, thus urinary bladder detrusor may be overactive in obstructive bladder with BPH.